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IN THE CLAIMS:

Please amend claims 1-24 as follows:

1. (Amended) A method of domain specific gene evolution of a target nucleic acid encoding an amino acid sequence of interest, said method comprising:

contacting a target nucleic acid with a recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid encoding a first domain of a polypeptide, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence, to form a first library of altered target nucleic acids; and repeating said contacting on said library of altered nucleic acids.

2. (Amended) A method according to claim 1, further comprising:

contacting said target nucleic acid with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

3. (Amended) A method of domain specific gene evolution comprising:

a) contacting a target nucleic acid encoding a polypeptide of interest with a recombinase and a first pair of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said nucleic acid encoding a first domain of said polypeptide, to form a first recombination intermediate;

b) contacting said recombination intermediate with a nuclease to form a nicked or open ended target nucleic acid; and

c) reassembling and recombining said nicked or open ended target nucleic acid to evolve a first library of altered target nucleic acids.

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4. (Amended) A method according to claim 3 further comprising:

d) combining said target nucleic acid with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

5. (Amended) A method of generating a library of altered nucleic acid sequences of a pre-selected target nucleic acid sequence in an extrachromosomal sequence, said method comprising:

a) contacting an extrachromosomal nucleic acid comprising a target nucleic acid sequence with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein

each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to evolve a first library of altered target nucleic acids; and

b) repeating step a) on said library of altered target nucleic acids.

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6. (Amended) A method according to claim 5 further comprising:

c) adding to said extrachromosomal nucleic acid a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotide and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

7. (Amended) A method of generating a library of altered nucleic acids of a pre-selected target nucleic acid in a chromosomal sequence, said method comprising:

a) contacting a chromosomal nucleic acid comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to form a first library of altered target nucleic acids; and

b) repeating step a) on said library of altered target nucleic acids.

8. (Amended) A method according to claim 7 further comprising:

c) adding to said chromosomal nucleic acid a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

9. (Amended) A method according to any one of claims 1, 2, 3, 4, 25, 26, 27, and 28 further comprising repeating said method on said library of altered target nucleic acids.

10. (Amended) A method according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, and 28 further comprising introducing said library of altered target nucleic acids into cells to form a cellular library comprising variant nucleic acid sequences.

11. (Amended) A method according to claim 10 further comprising expressing said library of altered target nucleic acids to generate a library of variant polypeptides.

12. (Amended) A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid having a desired activity.

13. (Amended) A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid and having a desired phenotype.

14. (Amended) A method according to claim 11 further comprising secreting said library of variant amino acid sequences.

15. (Amended) A method according to claim 10 wherein said recombinase is removed prior to said introducing.

16. (Amended) A method according to claim 29 wherein said cell is eukaryotic.

17. (Amended) A method according to claim 29 wherein said cell is procaryotic.

18. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said targeting polynucleotides are coated with said recombinase.

19. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said recombinase is a species of prokaryotic recombinase.

20. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said recombinase is a species of eukaryotic recombinase.

21. (Amended) A method according to claim 11, wherein said variant polypeptides comprise a plurality of amino acid substitutions.

22. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein at least one of said targeting polynucleotides further comprises a chemical substituent.

23. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said target amino acid comprises a complementary determining region.

24. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said target nucleic acid comprises an expression vector.

Please add the following new claims:

25. (New) A method according to claim 1, further comprising:

contacting all or part of said first library of altered nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

26. (New) A method according to claim 3 further comprising:

d) contacting said first recombination intermediate with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

27. (New) A method according to claim 5 further comprising:

c) contacting all or part of said first library of altered target nucleic acids with at least one

recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

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wherein said repeating is on said second library of altered target nucleic acids.

28. (New) A method according to claim 7 further comprising:

c) contacting all or part of said first library of altered target nucleic acids with at least one recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

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29. (New) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 further comprising contacting said recombination intermediate with a recombination proficient cell.

REMARKS

Claims 1-29 are pending. Support for the amendments is found in the claims as filed, and in particular as follows. Further support for the amendments to claims 1-2 can be found at p.4,